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Effects of Reducing the Ambient UV-B Radiation in the High Arctic on *Salix arctica* and *Vaccinium uliginosum*.

By

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K e y w o r d s : UV-B, high arctic, chlorophyll-a fluorescence, fluorescence transient, photosynthesis, flavonoids.

S u m m a r y

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Effects of reducing the ambient UV-B radiation on gas exchange and chlorophyll fluorescence of two dwarf shrub species, *Salix arctica* and *Vaccinium uliginosum*, was studied in a high arctic heath in North East Greenland during two growing seasons. Films (Mylar, transmitting $\lambda > 320$ nm, and Lexan, transmitting $\lambda > 400$ nm) were used to reduce UV-B radiation and UV-B+A respectively. A UV transparent film (Teflon, transmitting $\lambda > 280$ nm) and no film were used as controls. Field measurements showed that the plants under Teflon, Mylar and Lexan received app. 91%, 39% and 17% of the ambient UV-B irradiance, respectively. UV radiation decreased the maximal photochemical efficiency (F_v/F_m) and other fast fluorescence transient derived parameters in both species, despite an increased level of leaf flavonoid content. The responses varied in significance according to species and site. The relation of these effects to a significantly decreased stomatal conductance (g_s) and intercellular CO₂ concentration (C_i) pointed to respiration as an important factor in the interpretation of the observed unaffected net CO₂ assimilation (P_n) in UV-reduced treatments. It is concluded that the studied species have not fully acclimatized to the level of ambient UV-B radiation, and that ambient UV-B level is an important stress factor for the investigated plants in High Arctic.

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Introduction

High Arctic plants live at the limit of their distribution in an extreme environment with a short growing season, low temperature, and often nutrient limitation. Moreover, the longevity of arctic plants makes them adapt only slowly (CALLAGHAN & JONASSON 1995), for which reason acclimation is of special importance when facing changes in the environment, e.g. UV-B radiation (CALDWELL & al. 1980, ROBBERECHT & al. 1980). Depletion of stratospheric ozone in Arctic regions is followed by an increase in the UV-B radiation (280 to 315 nm) in the biosphere (e.g. MADRONICH & al. 1998). Currently, the UV-B irradiance level in the arctic region is considered to be near its maximum and the ozone column is estimated to recover towards the middle of the century (WMO 2003). The relative increase in UV-B irradiance has been occurring most rapidly at high latitudes and also the absolute net depletion of ozone has been highest, why the potential highest impact on the vegetation is expected there (BJÖRN & al. 1999, PAUL 2001). For a critical view see ALLEN & al. 1998.

To investigate possible effects of ambient UV-B on high arctic vegetation a manipulative study was performed, where screening by various filters reduced the UV-B radiation load on the vegetation, as done by others (HUSKIES & al. 2001, DAY & al. 2001, ROBSON & al. 2003), excluding the spectral matching problems of natural irradiation as experienced in many earlier UV-B supplementation experiments (CALDWELL & FLINT 1994, SEARLES & al. 2001). This setup allowed us to investigate the effects of reducing the present UV-B irradiance received by high arctic vegetation on different scales and to study possible seasonal variations in these effects. In general it was hypothesised that arctic plants may be negatively influenced by the ambient UV-B level and if the vegetation has not been fully acclimated to the present UV radiation, reductions of the irradiance load would improve the photosynthetic performance of the plants.

Material and Methods

Experimental site, setup and treatments

The fieldwork was carried out in a high arctic heathland at Zackenberg Research Station, North East Greenland (74°N; 21°E), in July and August 2001 and 2002. The plant species investigated were the frequent occurring *Salix arctica* Pall. and *Vaccinium uliginosum* L., ssp. *microphyllum* Lge., having flat leaves well suited for physiological measurements. The aim was to establish plots where parts of the UV spectrum in natural daylight were reduced with ambient UV irradiance as reference. Reductions were achieved by filtering the solar radiation through two different filters, Mylar® (type D, DuPont Teijin Films, Wilmington, Delaware, USA) and Lexan® (RIAS, Roskilde, Denmark) transmitting $\lambda > 320$ nm and $\lambda > 400$ nm, respectively. As control was used a Teflon® filter (Fluoretek AB, Knivsta, Sweden.) transmitting $\lambda > 280$ nm. A treatment with no filter was also established. Measurements in the experimental area with a broad-band cosine corrected UV-B sensor (UV-S-310-T, Scintec – now UV-S-B-T, Kipp & Zonen B.V., Delft, The Netherlands) showed that the plant canopy under Teflon, Mylar and Lexan were exposed to app. 91%, 39% and 17% of the clear sky UV-B irradiance, respectively, slightly depending on the exposure angle to the sun (BREDAHL & al. 2004). The 17% UV-B measured under Lexan and a percentage of the clear sky

UV-B irradiance in the other treatments are due to diffuse and reflected radiation reaching the UV-B sensor from the open sides not covered with filters.

In 2001 two sampling sites were chosen, in the following named site 1 and 2, and filters were placed parallel to the soil surface 5 cm above canopy by means of 40 cm x 60 cm aluminium frames. The experiment was designed as a randomised block design where each site consisted of four experimental plots (replicates) of each treatment, in total 16 plots per site. Both sites were south facing slopes, chosen to maximise the incoming amount of radiation and to make possible that the plots could benefit from uphill precipitation. Site 1 was marginally sloping (app. 5 degrees) while the inclination of site 2 was much steeper (app. 45 degrees).

In 2002 a third site were established, in the following named site 3. Here rectangular aluminium frames were provided with nylon strings enabling fixation of plant shoots and leaves. Each frame was forced into the soil just in front of a *Salix arctica* plant base. Then one long (10-20 cm) shoot was carefully fixed such that all leaf surfaces were exposed in an angle of 45° facing south. Then filter sheets of Mylar® or Teflon® were fastened to the frames with clamps. This setup exposed the plants to maximum PAR and UV-B and allowed easy access for measurements. This experiment was also a randomised design where each treatment consisted of 20 shoots from different plant individuals, in total 40 shoots.

Measurements

Net photosynthesis (P_n), calculated from leaf gas exchange, was measured weekly at site 1 on *Salix arctica*. Maximal photochemical efficiency, determined from chlorophyll fluorescence (F_v/F_m), was measured on site 1 and 2 every third day on *Salix arctica* and weekly on *Vaccinium uliginosum* leaves.

Leaf gas exchange was measured with a CIRAS-1 connected to an automatic broad leaf cuvette (PLC(B)) (PP Systems, Hertfordshire, UK) at leaf temperature optimum (20°C) and saturating photon irradiance (1200 $\mu\text{mol}/\text{m}^2/\text{s}$) at two CO_2 levels: Ambient (364 ppm) and saturated (1800 ppm). The gas exchange measurements were done between 11 a.m. and 10 p.m. The time of the day did not influence P_n (data not shown). Possible plot edge effects were taken into account by preferentially avoiding measurements on leaves near the edges of the plots.

Transients of chlorophyll-a fluorescence were recorded and digitized with a portable Handy PEA (Hansatech Instruments, Ltd. King's Lynn Norfolk, UK) on the leaves after a dark adaptation period of 35-40 min. On site 1 and 2 measurements on *Salix arctica* were done *in situ* whereas leaves of *Vaccinium uliginosum* were excised and immediately brought to the laboratory to be measured. This procedure was tested, and the detachment did not affect the response. During two days in August series of measurements were performed, where F_v/F_m and gas exchange were measured on the same *Salix arctica* leaf consecutively in order to compare the two physiological parameters directly on leaf basis. On site 3 transients of chlorophyll-a fluorescence were measured *in situ* approximately every third day at 14.30 am on *Salix arctica*. Initial measurements were done at July 6th on the plants after their fixation, but before attachment of filters. Daily variation was investigated by measurements on August 1st, 6th and 13th, at 9:00 am, 14:00 am and 18:00 am. From the site 3 measurements a range of new parameters was calculated from the fluorescence transient (i.e. ABS/CS, TRo/CS, ETo/CS, DIo/CS, RC/CS and Performance Index) according to STRASSER & al. 2004. See ALBERT & al. 2005 for a throughout discussion. After the last measurements all leaves were harvested in order to determine leaf biomass, leaf area, and the content of flavonoids, C, and N. See ALBERT & al. 2005 and BREDAHL & al. 2004 for further details on sites and measurements.

Statistical analysis

Statistical analyses were conducted using the GLM procedure (SAS INSTITUTE 2002). Levene's test was used to test for homogeneity of variance. Where necessary parameters were transformed in order to meet the assumptions of ANOVA. All values presented here are non-transformed. In cases of significant treatment effects these analyses were followed by tests of treatment differences using Tukey's test. Differences are considered at the $P < 0.05$ level. In the following, analyses are referred to as "effect of treatments" when the tests included all four treatments,

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"effect of UV reduction" when the test included all filters (UV-B, UV-B+A and filter control) and "effect of UV-B reduction" when only UV-B and filter control were included in the tests.

Results

The analysis of the chlorophyll fluorescence measurements at site 1 and 2 revealed an overall effect of treatment (all $P < 0.0001$) and of UV reduction on F_v/F_m (all $P < 0.0001$ at site 2; $P = 0.0002$ for *Salix arctica* and $P = 0.0023$ for *Vaccinium uliginosum* at site 1). The values of F_v/F_m in the filter control were somewhat higher than 0.7, indicating a generally low stress level in the leaves of the two species. The F_v/F_m levels at the two sites were very similar but aided by the large number of samplings ($n = 6896$), the overall F_v/F_m proved to be significantly lower at site 2 than at site 1 ($P < 0.001$). At site 1 the overall F_v/F_m did not differ significantly between the two species but at site 2 *Vaccinium uliginosum* had a significantly lower overall F_v/F_m ($P < 0.001$).

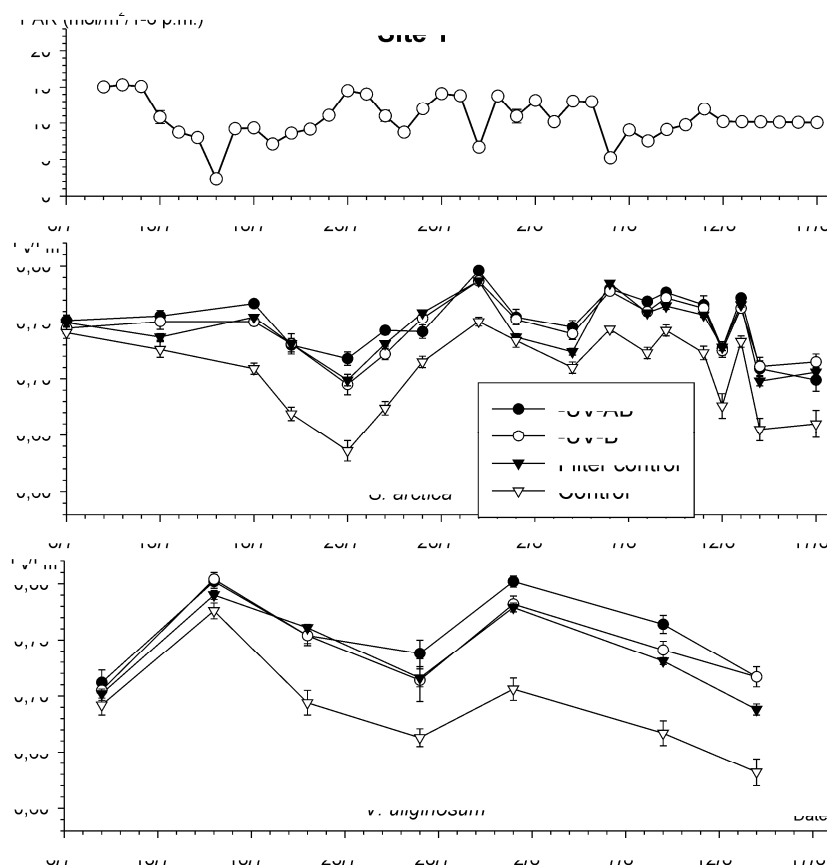


Fig. 1. Mean F_v/F_m (+/- 1 SE) per treatment per sampling day and cumulated PAR between 1-6 p.m. at site 1 showing when significant differences between the filter treatments were found (2-factor ANOVA, filter treatments only). Days with significance are marked with * when $P < 0.05$; ** when $P < 0.01$ or *** when $P < 0.001$. Data from BREDAHL & al. 2004.

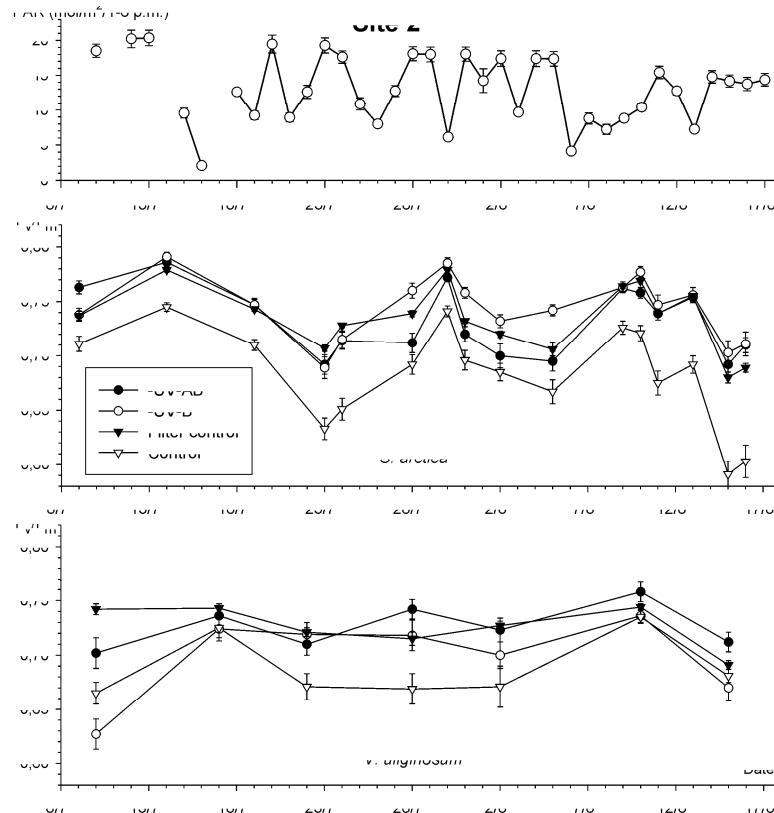


Fig. 2. Mean F_v/F_m per treatment per sampling day and cumulated PAR at site 2. See fig. 1 legend. PAR data on July 11, 14 and 17 are omitted because of errors. Data from BREDAHL & al. 2004.

Fig. 1 and Fig. 2 show the seasonal variation in F_v/F_m on site 1 and 2 respectively. For *Salix arctica* the effects were pronounced in the middle of the sampling period with values of F_v/F_m being significantly higher after UV-B+A reduction at site 1 and after UV-B reduction at site 2. For *Vaccinium uliginosum* significantly positive effects of mainly UV-B+A reduction was seen on F_v/F_m in the end of the growing season at site 1 (higher values). Mean values of F_v/F_m correlated negatively with midday PAR (1- 6 p.m.) at each site ($r = -0.80$, $P < 0.0001$, $n = 20$ and $r = -0.74$, $P = 0.001$, $n = 16$ at site 1 and 2, respectively).

At site 3 after the initial measurement July 6th, F_v/F_m was significantly reduced throughout July in ambient UV-B ($P < 0.0041$), but from Mid-august, the treatment effects disappeared (Fig. 3). Data from August 1st, 6th, and 13th were pooled and the daily variations in fluorescence variables were tested for time and treatment effects.

No significant effects due to treatment were found. The time effects were examined as the difference between 9:00 am and 14:00 am (midday) and the difference between 14:00 am and 18:00 am (afternoon). Both at midday and in the after-

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noon F_v/F_m was unaffected, but all other derived parameters clearly demonstrated a pronounced midday depression (ALBERT & al. 2005).

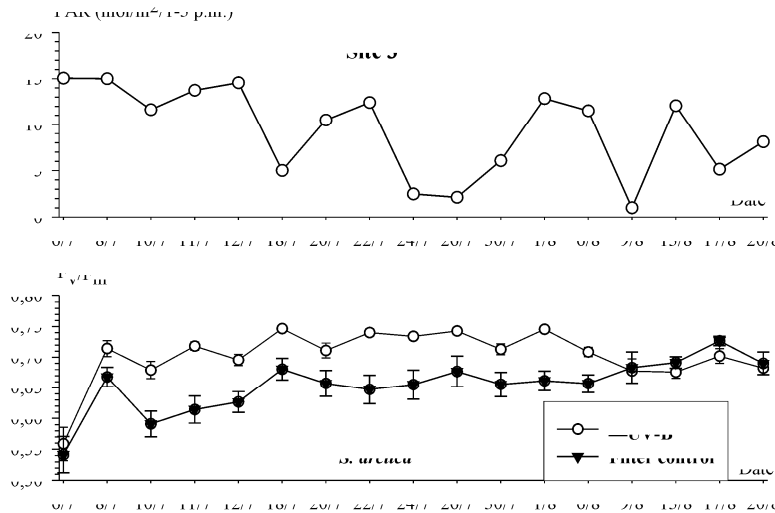


Fig. 3. Mean F_v/F_m (± 1 SE) per treatment per sampling day and cumulated PAR between 1-3 p.m. at site 3 showing when significant differences between the filter treatments were found (1-factor ANOVA, filter treatments only). See fig. 1 legend. Data from ALBERT & al. 2005.

The analysis of the CO_2 gas exchange measurements revealed no significant influence of UV reduction on the overall mean values of P_n of *Salix arctica*. Despite this, both UV reduction treatments resulted in significantly lower overall values of stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) (all $P < 0.002$). This was the case at both ambient and saturating CO_2 level. Apparently C_i did not show any particular seasonal variation whereas g_s showed same seasonality as P_n . From the simultaneous measurements on the same *Salix arctica* leaves, a correlation between P_n and F_v/F_m (all treatments) was found when P_n was measured at ambient CO_2 level ($r = 0.35$, $P = 0.006$) but not at saturating level (BREDAHL & al. 2004).

Leaf biomass and leaf area at site 3 were lower in ambient UV-B, but not significantly ($P < 0.2387$ and $P < 0.4866$). Also, a higher, but not significant, specific leaf area (SLA) in ambient UV-B was found ($P < 0.2238$). No significant differences were found for C, N, and C/N ratio (ALBERT & al. 2005). Across site 1 and 2, for both *Vaccinium uliginosum* and *Salix arctica*, the flavonoid content was significantly different between treatments ($P < 0.0365$), and the levels were decreasing in the order $C > F > B > AB$ (K.A.ALBERT, unpublished), which follows the increasing UV-B screening in the treatments. Also on site 3 a higher flavonoid content were found in leaves exposed to ambient UV-B ($P < 0.0001$) (ALBERT & al. 2005).

Discussion

Few studies of effects of UV-B exclusion in the High Arctic have been performed, and in contrast to other UV-B exclusion experiments in natural vegetation at high latitudes (XIONG & DAY 2001, LUD & al. 2001) these results demonstrates significantly higher values of maximal photochemical efficiency, F_v/F_m , at reduced UV levels. This was seen despite the significantly higher content of flavonoids, which in general are expected to protect PSII due to their UV-B absorbing and antioxidant characteristics (e.g. TEVINI & TERAMURA 1989). The higher SLA in ambient UV-B, although not significant, might also indicate a UV-B screening response as found by others (e.g. MEIJKAMP & al. 2001), but insufficient in this particular case. Although large variability was seen in the level of F_v/F_m , a general decrease was observed at high PAR at site 1 and 2. This variability were much lower on site 3 due to the leaf angle control making the radiation dose more equal. These results indicates that the lower levels of F_v/F_m on site 2 and the even lower levels at site 3 are caused by the higher PAR irradiance at these sites. Further, a clear mid-day depression were seen in most chlorophyll fluorescence variables at site 3 (ALBERT & al. 2005).

In response to the increased production of UV-B absorbing compounds and the decreased F_v/F_m , it could in theory be expected that maintenance respiration would increase due to repair of PSII damage and increased flavonoid synthesis. As a result, the supply of electrons to the Calvin Cycle would be lowered due to decreased PSII activity or damage. These mechanisms may alone, or in combination, have a negative impact on P_n . The significant, but weak, correlation of F_v/F_m and P_n might indicate that this is possible. In this study a significantly unaffected P_n was found, however, both g_s and C_i were significantly higher in the filter control plots (near-ambient UV-B) on site 1 and 2.

Direct effects of UV-B on the guard cells preventing them to close properly have been reported from UV-B supplemental experiments (NOGUÉS & al. 1999, COOLEY & al. 2000), but other experimenters have shown that supplemental UV-B resulted in reduced g_s (CORREIA & al. 1999, NOGUÉS & al. 1999). In contrast to these findings, this study found an increased g_s followed by a similar increase in C_i in ambient UV-B. As more closed stomates make changes in C_i more dependent on internal CO_2 exchanges among the mesophyll cells, we believe that the reduced C_i observed is more determined by effects on respiration and/or rates of carboxylation/oxygenation of RuBisCO rather than being a direct result of a reduced g_s . This is in accordance with the absence of effects on P_n , which, in case of C_i having been determined only by g_s , would as stated above be expected to have decreased too. Actually, a decreased net photosynthesis were found on canopies of *Vaccinium uliginosum* in ambient UV-B on a nearby site in 2002 ($P < 0.0001$) (K.R. Albert, unpublished), but due to the experimental setup g_s was not determined. UV-B effects on respiration is not well investigated (ROZEMA & al. 1997), why this treatise points to the need for further research concerning this.

From the studies presented here it is concluded that ambient UV-B is a potential important stress factor in the High Arctic. Considering that accumulating

effects of ambient UV-B have been found in Antarctic (DAY & al. 2001, ROBSON & al. 2003) and that a further increase of the ambient UV-B level is presumed (WMO 2003), there is a need for continued experiments to investigate the importance of the long term effects of ambient UV-B on the vegetation at high latitudes. This might reveal if High Arctic plant species are adequately acclimatized to the ambient UV-B radiation, but within this treatise the investigated species are concluded not to be fully acclimatized to the present ambient UV-B radiation.

A c k n o w l e d g e m e n t s

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